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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Emanuel Calenoff and Charles Ditlow

Serial No.: 09/848,967

Examiner: NA

Attorney Docket No. 21417/92378

Art Unit: 1647

Filing Date: May 4, 2001

Title: IMMUNOGENIC PEPTIDES AND USES THEREOF

PRELIMINARY AMENDMENT TO ACCOMPANY SEQUENCE LISTING IN ACCORDANCE WITH 37. C.F.R. § 1.821-1.825

Assistant Commissioner of Patents
BOX MISSING PARTS
Washington, D.C. 20231

Dear Sir:

Please enter the following amendments:

In the Specification

On page 10, line 27, please add --(SEQ ID NO:1) after "sequence"

On page 10, line 32, please add --(SEQ ID NO:3)-- after "*Helicobacter pylori*"

On page 10, line 33, please add --(SEQ ID NO: 2)-- after "*Streptococcus pneumoniae*"

On page 10, line 34, please add --(SEQ ID NO:4)-- after "protein"

On page 11, line 7, please add --(SEQ ID NO: 3)-- after "peptide sequence"

On page 11, line 10, please add --(SEQ ID NOS: 2 & 4)-- after "sequences"

On page 11, line 17, please add --(SEQ ID NO: 5)-- after "MQEIDKKLTQKN"

On page 11, line 25, please replace "QKDAKELKGKRN" with --KNLESYQKDA (SEQ ID NO:6)--

On page 11, line 34, please add --(SEQ ID NO:7)-- after "QKDAKELKGKRN"

On page 12, line 5, please add --(SEQ ID NOS· 8-19, respectively, in order of appearance)-- after "antigens"

On page 12, line 11, please add --(SEQ ID NOS: 20-32, respectively, in order of appearance)-- after "antigens"

On page 13, line 8, please add --(SEQ ID NO: 5)-- after "MQEIDKKLTQKN"

On page 13, line 9, please add --(SEQ ID NO: 6)-- after "KNLESYQKDA"

On page 13, line 10, please add --(SEQ ID NO: 7)-- after "QKDAKELKGKRN"

In the FIGS

In FIG. 1, please add --(SEQ ID NO:1)-- after "**qknlesyqkdakelkgkrn**r"

In FIG. 2a, please add --(SEQ ID NO:2)-- after "homologous sequence"

In FIG. 2a, please add --(SEQ ID NO:3)-- after "protein sequence"

In FIG. 2a, please add --(SEQ ID NO:4)-- after "homologous sequence"

In FIG. 2b, please add --(SEQ ID NO:2)-- after "homologous sequence"

In FIG. 2b, please add --(SEQ ID NO:3)-- after "protein sequence"

In FIG. 2b, please add --(SEQ ID NO:4)-- after "homologous sequence"

In FIG. 3, please add --(SEQ ID NO: 5)-- before "Test Data"

In FIG. 4, please add --(SEQ ID NO:6)-- before "Data"

In FIG. 5, please add --(SEQ ID NO:7)-- before "Data"

In FIG. 6, please add --(SEQ ID NO:8)-- after "LPALKENNGKV"

In FIG. 6, please add --(SEQ ID NO:9)-- after "VTTKGERTFEYNN"

In FIG. 6, please add --(SEQ ID NO:10)-- after "LEQNEGFKRRV"

In FIG. 6, please add --(SEQ ID NO:11)-- after "TVDASGKRSISG"

In FIG. 6, please add --(SEQ ID NO:12)-- after "MWEIDKKLTQKN"

In FIG. 6, please add --(SEQ ID NO:13)-- after "NTRYDRWAKD"

In FIG. 6, please add --(SEQ ID NO:14)-- after "INGDKRTGGKPNTPE"

In FIG. 6, please add --(SEQ ID NO:15)-- after "SSSEYEKLKA"

In FIG. 6, please add --(SEQ ID NO:16)-- after "KEQIIEAKGPDV"

In FIG. 6, please add --(SEQ ID NO:17)-- after "QQTHRKINRP"

In FIG. 6, please add --(SEQ ID NO:18)-- after "KQAEEANKTP"

In FIG. 6, please add --(SEQ ID NO:19)-- after "KTPDKPDKVW"

In FIG. 8, please add --(SEQ ID NO:20)-- after "QGQDVRQPG"

In FIG. 8, please add --(SEQ ID NO:21)-- after "GRDGE"

In FIG. 8, please add --(SEQ ID NO:22)-- after "GGFDEKAGGA"

In FIG. 8, please add --(SEQ ID NO:23)-- after "WPGLPGA"

In FIG. 8, please add --(SEQ ID NO:24)-- after "GLPGTPGTDGPKGASGPAGPPGAQGPPG"

In FIG. 8, please add --(SEQ ID NO:25)-- after "GPEGAPGKDGGRGLT"

In FIG. 8, please add --(SEQ ID NO:26)-- after "GEVGPPGPAGSAGARGAP"

In FIG. 8, please add --(SEQ ID NO:27)-- after "TGPKGARGAQGPPGAGFPGAA"

In FIG. 8, please add --(SEQ ID NO:28)-- after "NGNPGPPGPPGPS"

In FIG. 8, please add --(SEQ ID NO:29)-- after "LQGPAGPP"

In FIG. 8, please add --(SEQ ID NO:30)-- after "AVGAPGAPGPPGSPGPAGPGKQGD"

In FIG. 8, please add --(SEQ ID NO:31)-- after "KGHRGFTGLQ"

In FIG. 8, please add --(SEQ ID NO:32)-- after "IGPPGPRGRSGETGPAGPPGN"

No fees in addition to that for the extension of time are believed due at this time, however, please charge any deficiencies or credit any overpayments to deposit account number 10-0435 with reference to our attorney docket number (21417/92378).

Please contact applicant's representative if there are any issues to be resolved.

Respectfully submitted,

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Attorney for Applicant

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MARKED UP VERSION OF SPECIFICATION
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Pages 10-12

FIG. 1 illustrates the amino acid sequence - -(SEQ ID NO: 1)- - of the flagellar sheath adhesion protein of *Helicobacter pylori*; the bolded and underlined portions of the sequence are portions that are hydrophilic and likely to be expressed on the surface of the folded protein.

FIG. 2a shows one of the hydrophilic peptide regions of the flagellar sheath adhesion protein of *Helicobacter pylori* - -(SEQ ID NO: 3)- - in alignment with closely matched peptide sequences of two comparative microoganismal proteins, the *Streptococcus pneumoniae* - -(SEQ ID NO: 2)- -, pspA protein and the *Mycoplasma hominis* Lp1 protein - -(SEQ ID NO: 4)- -; the pspA and Lp1 proteins were those most closely matching the linear amino acid sequence of the *Helicobacter pylori* flagellar sheath adhesion protein sequence using the BLAST amino acid sequence homology comparison program on the National Library of Medicine web site [www.ncbi.nlm.nih.gov:80/BLAST/]; only amino acids that are identical to *H. pylori* protein sequence, are shown.

FIG. 2b shows three boxes drawn around different constituent sub-sequences of the *Helicobacter pylori* flagellar adhesion sheath peptide sequence- -(SEQ ID NO: 3)- -; the *Helicobacter pylori* amino acid sequence with the bold lined box is likely to serve as a functionally specific antigen when compared to the two aligned, comparative protein amino acid sequences - -(SEQ ID NOS 2 & 4)- - using the selection criteria of the disclosed invention; results using a peptide with this sequence shown in FIG. 3; the *Helicobacter pylori* sequence within the second, lightly lined box also satisfies the selection criteria of the present invention; results using a peptide with this sequences are shown in FIG. 4; whereas the *Helicobacter pylori* sequence within the third, dashed line box does not satisfy the selection criteria and would be discarded or rejected as a candidate; results using this are shown in FIG. 5.

FIG. 3 is a graphical representation of results of using the *Helecobacter pylori* peptide sequences MQEIDKKLTQKN - -(SEQ ID NO: 5)- - shown in FIG.2b as a source antigen peptides used to complex with antibody in sera from 30 *Helicobacter pylori* infected patients and from 30 healthy control subjects; the dotted line at the bottom of the plotted results represents the positive/negative threshold of the immunoassay using the control mean plus 2/5 standard deviations; this peptide identified three *Helicobacter pylori* infected individuals from within a

group of thirty; no control sera were incorrectly identified as positive for the peptide as determined by antibodies to the peptide.

FIG. 4 is a graphical representation of results of using the *Helicobacter pylori* peptide sequence[QKDAKECKGKRN] - -KNEESYQKDA (SEQ ID NO: 6)- - shown in FIG. 2b as a source peptide antigens used to complex with antibody in sera from 30 *Helicobacter pylori* infected patients and from 30 healthy control subjects; the dotted like at the top of the plotted results represents the positive/negative threshold of the immunoassay using the control mean plus 2.5 standard deviations; this peptide does not serve to identify *Helicobacter pylori* infected individuals from within a group of thirty in spite of satisfying most of the selection criteria of the described invention, thus confirming the need to test specific functional utility (immunogenic) of the peptide antigen.

FIG. 5 is a graphical representation of results of using the *Heicobacter pylori* peptide sequence QKDAKELKGKRN - -(SEQ ID NO: 7)- - shown in FIG. 2b as a source antigen used to complex with antibody in sera from 30 *Helicobacter pylori* infected patients and from 30 healthy control subjects; the dotted line at the center of the plotted results represents the positive/negative threshold of the immunoassay using the control mean plus 2.5 standard deviations; as expected, this peptide does not serve to identify *Helicobacter pylori* infected individuals from within a group of thirty because its structure fails the basic selection criteria of the present invention.

FIG. 6 lists additional functionally specific *Helicobacter pylori* antigens - -(SEQ ID NOS 8-19, respectively, in order of appearance)- - which satisfy all of the criteria of the present invention; these peptides were derived from different *H. pylori* targeted proteins shown in FIGS. 2a and 2b.

FIG. 7 summarizes the diagnostic capability made possible by testing (a) patient; and (b) control sera against a plurality of 14 individual, specific peptides using immunoassays incorporating the peptides listed in FIG. 6.

FIG. 8 lists functionally specific collagen type II antigens - -(SEQ ID NOS 20-32, respectively, in order of appearance)- - which satisfy all of the listed criteria of the described invention; type II collagen is one of several collagen types known to be associated with rheumatoid arthritis (He, 2000; Morgan,1987).

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Comparison of the aligned amino acid sequences were made for the targeted, and at least 2 of the comparative proteins. A sequence of at least 4 amino acids became a sequence for a candidate peptide that could be specific for *H. pylori*. Candidate peptides have sequences that are capable of immunologically distinguishing biological samples from diseased vs. non-diseased persons. For example, sequence MQEIDKKLTQKN - ~~(SEQ ID NO: 5)~~ - is a candidate sequence that was tested; results are shown in FIG. 3. Sequence KNLESYQKDA ~~(SEQ ID NO: 6)~~ is a candidate sequence that was tested, results are shown in FIG. 4. Sequence QKDAKELKGKRN ~~(SEQ ID NO: 7)~~ is a candidate sequence that was tested, results are shown in FIG. 5. As can be seen from the test results, the candidate sequences in FIGS. 4 and 5 were not functionally specific for the *H. pylori* protein.

Marked-Up Version

1 mktnghfkdfawkkcllgasvgallvgcsphiietnevalklnyhpasekvqaldekill

61 lrpafqysdniakeyenkfknqtvlkveqllqnqgykvinvdssdkddfsfaqkkeqyla

121 vamngeivlrpdpkrtiqkksepgllfstgldkmegvlipagfvkvtilepmsgesldsf

181 tmdlseldigekflktthsshsgglvstmvkqtdnsndaiksalnkifgsimqeidkklt

241 gknlesvqkdakelkgkrnr --(SEQ ID NO:1)--

FIG. 1

Streptococcus pneumoniae pspA homologous sequence -> --(SEQ ID NO:2)--	x x x I D K x x T Q K x L E x x x x D A x E L x x x x x
H. pylori flagellar sheath adhesion protein sequence -> --(SEQ ID NO:3)--	M Q E I D K K L T Q K N L E S Y Q K D A K E L K G K R N
Mycoplasma hominis Lp1 homologous sequence -> --(SEQ ID NO:4)--	x x E I x K K L x x x x x E S x x K D A K E L x G x R N

FIG 2a

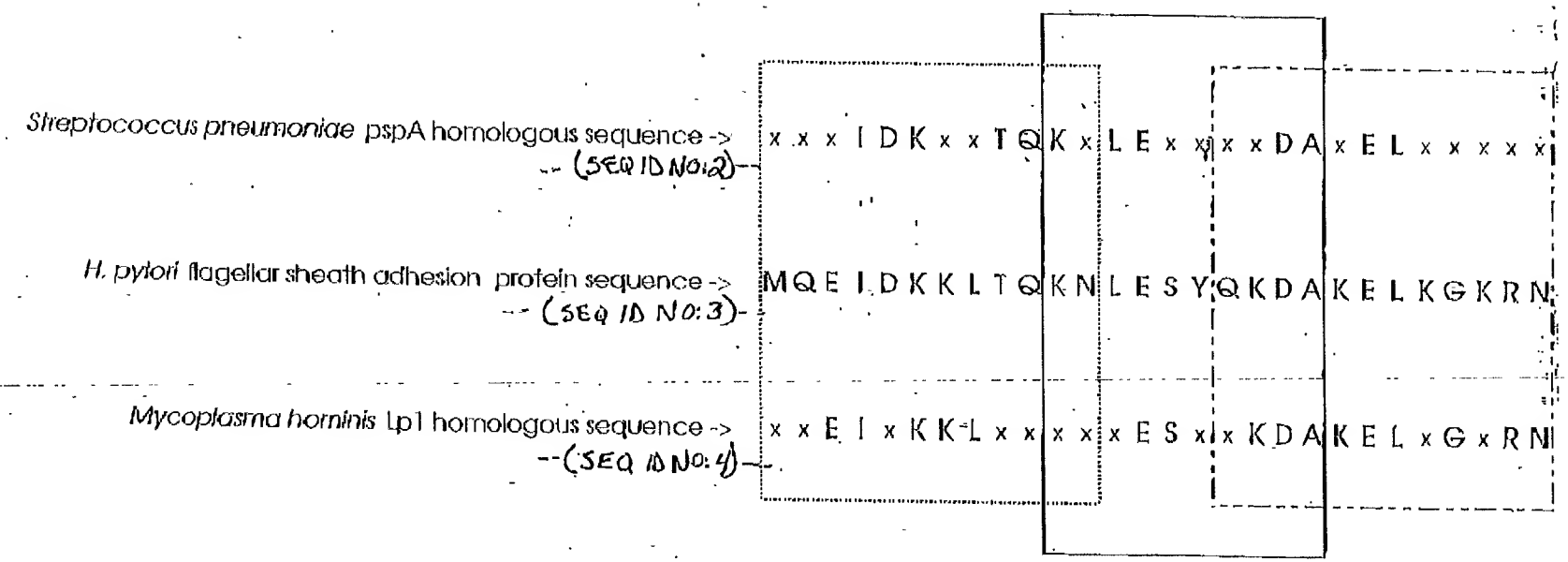

Figure 3 Plot

Figure 5 Plot

Figure 4 Plot

FIG 2b

FIG. 3

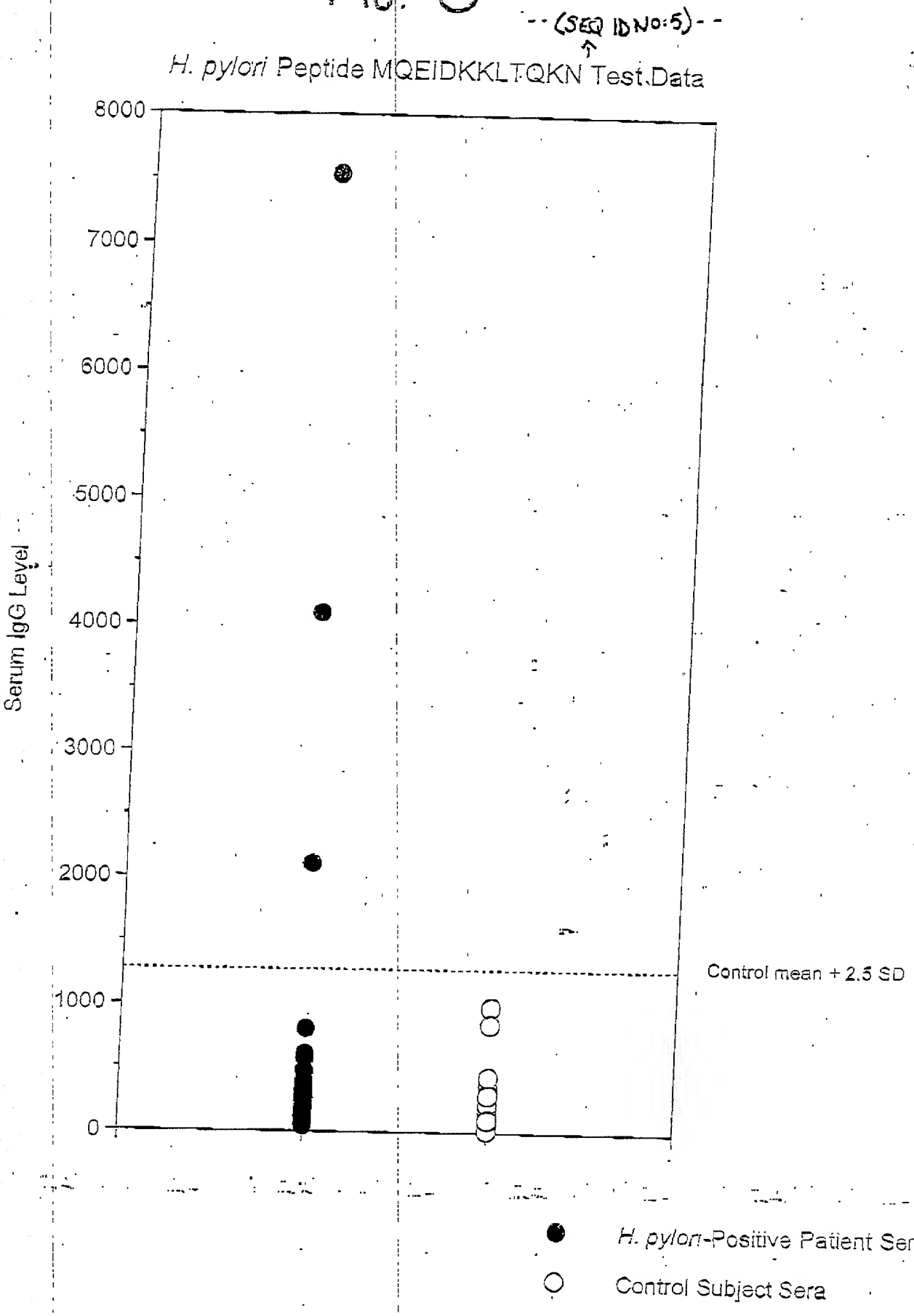
--(SEQ ID NO:5)--
H. pylori Peptide MQEIDKKLTQKN Test Data
8000
7000
6000
5000
4000
3000
2000
1000
0
Serum IgG Level
Control mean + 2.5 SD
H. pylori-Positive Patient Sera
Control Subject Sera

FIG 4

H. pylori Peptide KNLESYQKDA (SEQ ID NO:6) Data

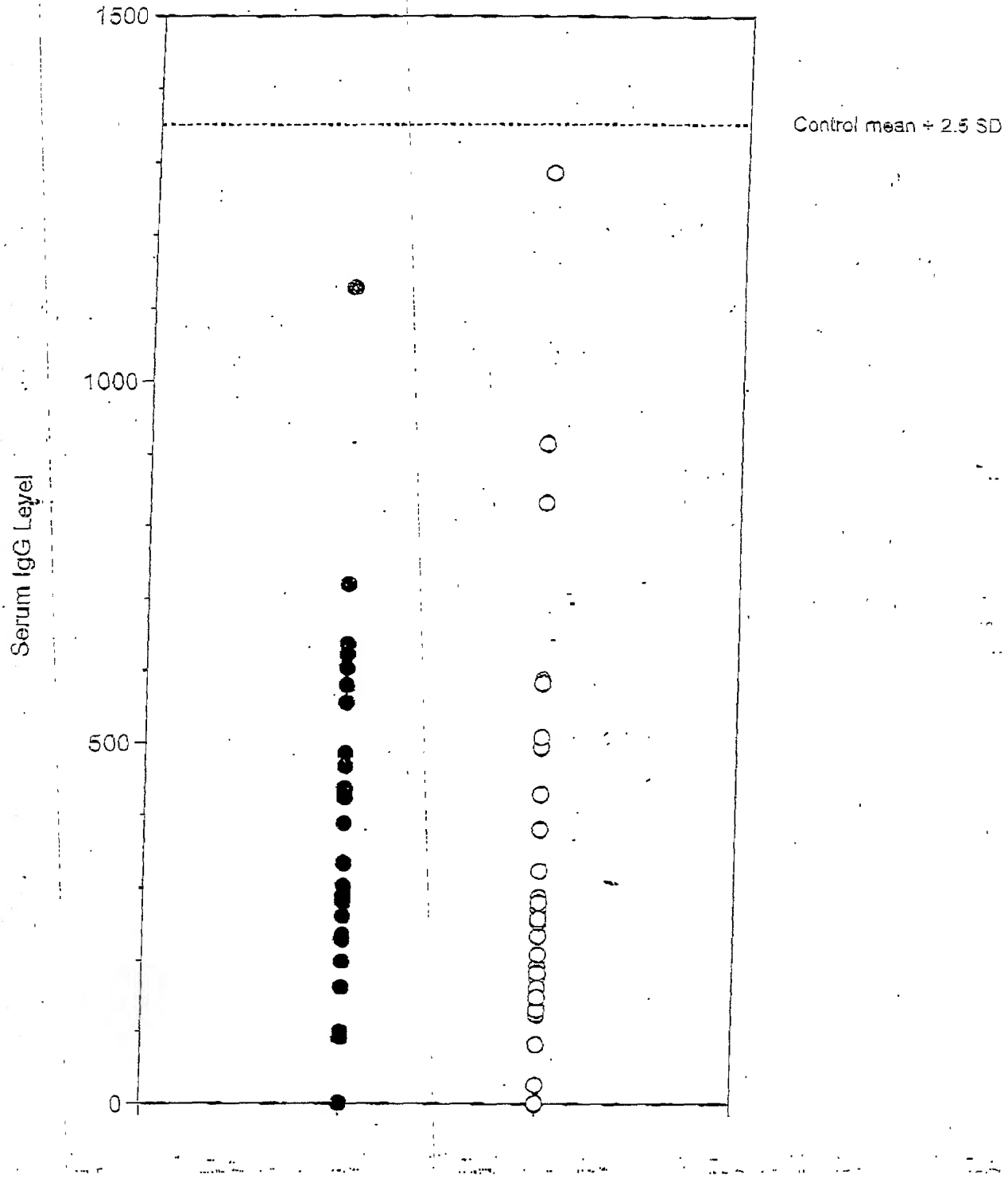

FIG 5

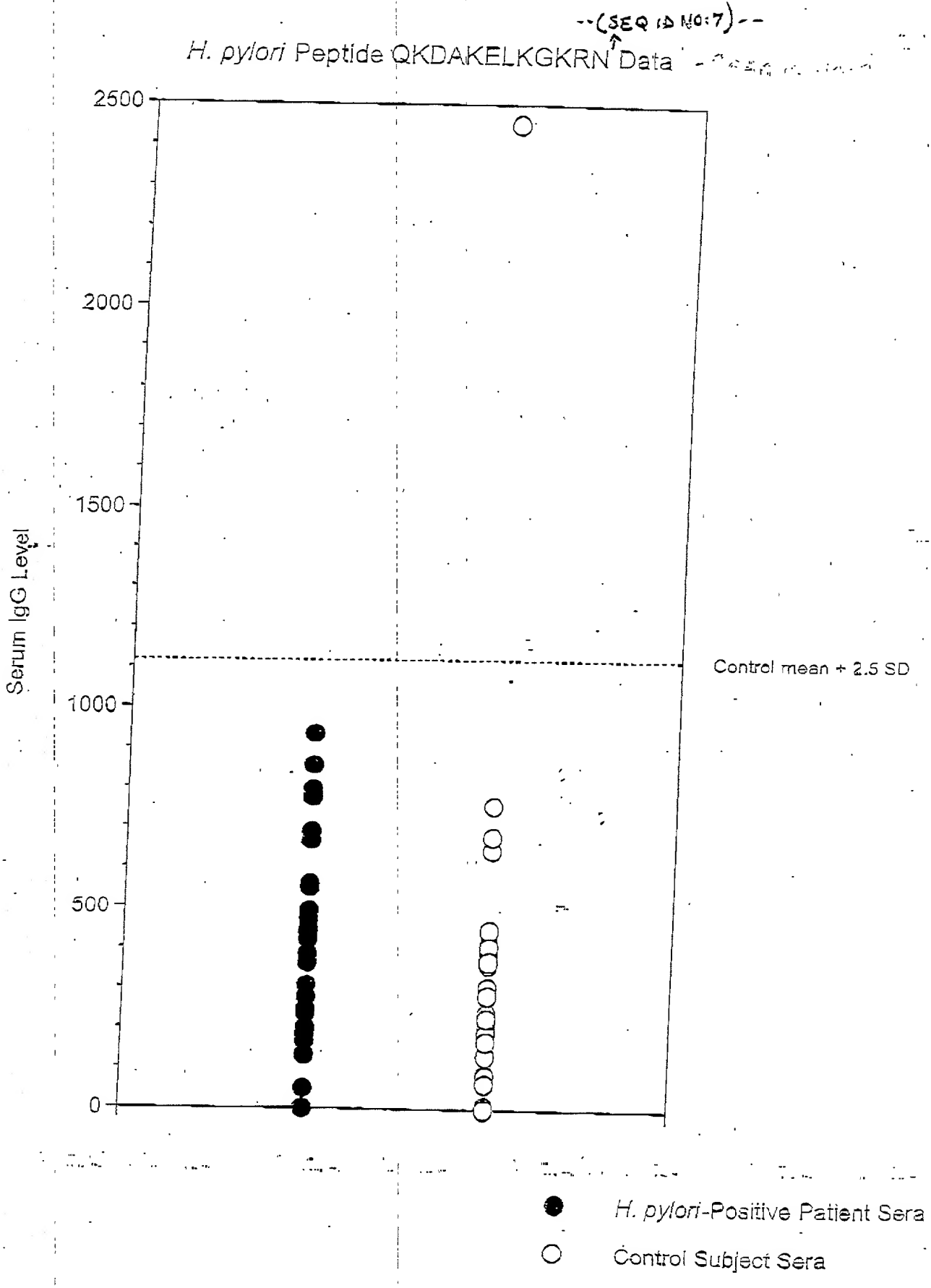
--(SEQ ID NO:7)--
H. pylori Peptide QKDAKELKGKRN Data
2500
2000
1500
1000
500
0
Serum IgG Level
Control mean + 2.5 SD
H. pylori-Positive Patient Sera
Control Subject Sera

FIG. 6

Parent *H. pylori* Protein	Functionally Specific Peptide Antigens
Adhesin 2	LPALKENNGKV--(SEQ ID NO:8)--
Iron-Regulated Outer Membrane Protein	VTTKGERTFEYNN --(SEQ ID NO:9)--
Lipid A Disaccharide Synthase	LEQNEGFKRRV--(SEQ ID NO:10)--
LPP20 Lipoprotein	TVDASGKRSISG --(SEQ ID NO:11)--
Flagellar Sheath Adhesin	MQEIDKKLTQKN --(SEQ ID NO:12)--
Outer Membrane Protein 1	NTRYDRWAKD --(SEQ ID NO:13)--
Outer Membrane Protein 19	INGDKRTGGKPNTPE --(SEQ ID NO:14)--
Outer Membrane Porin 1	SSSEYEKLKA --(SEQ ID NO:15)--
Outer Membrane Porin 2	KEQIIEAKGPDV --(SEQ ID NO:16)--
Vacuolating Cytotoxin	QQTHRKINRP --(SEQ ID NO:17)--
	KQAEEANKTP --(SEQ ID NO:18)--
	KTPDKPDKVW --(SEQ ID NO:19)--

Human Type II CollagenFunctionally Specific Peptide Antigens

QGQDVRQPG --(SEQ ID NO:20)--

GRDGE --(SEQ ID NO:21)--

GGFDEKAGGA--(SEQ ID NO:22)--

WPGLPGA --(SEQ ID NO:23)--

GLPGTPGTDGPKGASGPAGPPGAQGPPG --(SEQ ID NO:24)--

GPEGAPGKDGGRGLT --(SEQ ID NO:25)--

GEVGPPGPAGSAGARGAP --(SEQ ID NO:26)--

TGPKGARGAQGPPGATGFPGAA --(SEQ ID NO:27)--

NGNPGPPGPPGPS --(SEQ ID NO:28)--

LQGPAGPP --(SEQ ID NO:29)--

AVGAPGAPGPPGSPGPAGPTGKQGD --(SEQ ID NO:30)--

KGHRGFTGLQ --(SEQ ID NO:31)--

IGPPGPRGRSGETGPAGPPGN --(SEQ ID NO:32)--

FIG 8

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FIG. 1 illustrates the amino acid sequence (SEQ ID NO: 1) of the flagellar sheath adhesion protein of *Helicobacter pylori*; the bolded and underlined portions of the sequence are portions that are hydrophilic and likely to be expressed on the surface of the folded protein.

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FIG. 5 is a graphical representation of results of using the *Helcobacter pylori* peptide sequence QKDAKELKGKRN (SEQ ID NO: 7) shown in FIG. 2b as a source antigen used to complex with antibody in sera from 30 *Helicobacter pylori* infected patients and from 30 healthy control subjects; the dotted line at the center of the plotted results represents the positive/negative threshold of the immunoassay using the control mean plus 2.5 standard deviations; as expected, this peptide does not serve to identify *Helicobacter pylori* infected individuals from within a group of thirty because its structure fails the basic selection criteria of the present invention.

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